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IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF :

ATSUSHI SUZUKI ET AL : EXAMINER: COE, S. D.

SERIAL NO: 09/944,079 :

FILED: SEPTEMBER 4, 2001 : GROUP ART UNIT: 1651

FOR: AGENT FOR PREVENTING, IMPROVING OR TREATING HYPERTENSION :

DECLARATION UNDER 37 C.F.R. §1.132

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Now comes Atsushi Suzuki who deposes and states:

1. That I am a graduate of Shizuoka University and received my Master's degree in the year 1989.

2. That I have been employed by Kao Corporation for 13 years as an researcher in the field of biological science.

3. That the experimental data presented below were obtained by me or under my direct supervision and control.

4. The experimental data (Table 1) show that the administration of a combination of chlorogenic acid and an organic acid (Vitamin C) having a molecular weight ranging from 60-300 (the elected species) provides superior antihypertensive effects than the administration of chlorogenic acid or the organic acid (Vitamin C) alone. Table 1 also

shows the superior antihypertensive effects exerted by the combination of caffeic acid (a nonelected species) and the organic acid (Vitamin C).

5. Evaluation f the antihypertensive effect of the combinati n of chlorogenic acid and Vitamin C on rats.

Table 1: Change in systolic blood pressure at 1, 6, 12 and 24 hours after administration of control or test compositions.

	1h	6h	12h	24h	ANOVA
Control Group (saline)	-0.7 ± 2.1	-1.3 ± 1.0	-2.2 ± 1.1	-1.4 ± 1.8	
Test Group I (Vitamin C)	-0.9 ± 2.2	-1.0 ± 1.5	0.5 ± 1.7	-0.6 ± 2.2	
Test Group II (chlorogenic acid)	-1.7 ± 2.1	-6.7 ± 1.8	-6.8 ± 2.3	-3.0 ± 2.8	
Test Group III (caffeic acid)	-4.3 ± 2.5	-2.4 ± 1.9	1.4 ± 1.9	0.1 ± 3.1	
Test Group IV (chlorgenic acid + Vitamin C)	**-3.3 ± 2.9**	**-7.0 ± 1.0**	**-7.6 ± 3.2**	**-6.1 ± 1.5**	*
Test Group V (caffeic acid + Vitamin C)	-5.1 ± 2.1	-5.2 ± 2.6	-4.3 ± 1.6	-3.5 ± 1.8	*

a) results shown as mean value ± standard error (n = 3 to 6)

b) *: indicates a significant difference compared to Test Group I as measured by the ANOVA test at a significance level of 5%.

6. As shown in Table 1, the combination of chlorogenic acid and the organic acid (Vitamin C) produced an immediate effect on reducing high blood pressure (see e.g., values for 1 hour) and sustained this effect over a 24 hour period (see e.g., values for 6, 12 and 24 hours), as compared to the organic acid (Vitamin C) alone. Treatment with chlorogenic acid alone did not produce a significant immediate effect, nor one that was sustainable for 24 hours. Similarly, the combination of caffeic acid and an organic acid (Vitamin C) exhibited a

significant immediate and sustainable effect on systolic blood pressure, compared to caffeic acid or the organic acid alone.

7. The experimental data reported above in Table 1 were obtained using the following materials and methods.

8. Animal handling and blood pressure testing. The blood pressure of each 14 week old male spontaneous hypertensive rat ("SHR") was preliminarily, continuously measured for 5 days by means of a commercially available non-invasive sphygmomanometer (manufactured by Softlon Co.), thereby fully accustoming the rats to the sphygmomanometry, and an evaluation test was then started. All the rats were bred in a breeding chamber in a rat zone under conditions of a temperature of 25°C ± 1°C, a relative humidity of 55 ± 10% and a lighting time of 12 hours (from 7 a.m. to 7 p.m.).

9. Administration of control and test compositions. Physiological saline was administered to the Control Group. In Test Group 1, a solution containing Vitamin C (200 mg/kg) dissolved into physiological saline was administered. In Test Group 2, a solution with chlorogenic acid (50 mg/kg) dissolved into physiological saline was used. In Test Group 3, a solution with caffeic acid (50 mg/kg) dissolved into physiological saline was used. In Test Group 4, a solution with chlorogenic acid (50 mg/kg) and Vitamin C (200 mg/kg) dissolved into physiological saline was administered. In Test Group 5, a solution with caffeic acid (50 mg/kg) and Vitamin C (200 mg/kg) dissolved into physiological saline was administered. Oral administration of each solution was performed evenly in a quantity of 10 ml/kg.

10. Testing method. Each group contained 3 to 6 spontaneously hypertensive rats ("SHR") that were 15 weeks old.. The systolic blood pressure of a tail artery of each rat was

measured before administration of the control or test solution, and 1, 6, 12 and 24 hours after administration.

11. Statistical processing method. The test results obtained and shown in Table 1 were analyzed and expressed as the mean variation ratio (%) and standard error to conduct an analysis of variance based on recurrent measurements. A significance level was defined as at most 5%.

12. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

13. Further deponent saith not.

Atsushi Suzuki
(Signed)

2002/8/9
(Date)

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